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ABSTRACT

Although being a primary objective in the management of type 2 diabetes, optimal glycaemic control is difficult to achieve and usually not maintained over time. Type 2 diabetes is a complex pathology, comprising altered insulin sensitivity and impaired insulin secretion. Recent advances in the understanding of the physiological functions of incretins and their degrading enzyme dipeptidyl-peptidase (DPP)-4 have led to the 'discovery' of a new class of oral anti-diabetic drugs. Several DPP-4 inhibitors (or gliptins) with different chemical structures are now available. These agents inhibit the degradation of the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and hence potentiate glucose-dependent insulin secretion. DPP-4 inhibitors inhibit DPP-4 activity by almost 100% in vitro, maintaining a ≥80% inhibition throughout the treatment period in vivo, thus prolonging GLP-1 half-life, and significantly reducing HbA1c generally by -0.7 – 0.8% as well as fasting and post-prandial glycaemia. They are well-tolerated with no weight gain and few adverse effects, and, of particular interest, no increase in hypoglycaemic episodes. Although different by their chemical structure and pharmacokinetic properties, the DPP4 inhibitors currently available have proven similar glucose lowering efficacy.

INTRODUCTION

Diabetes mellitus is recognized as a major health problem affecting millions of people and predisposing to micro- and macro-vascular complications including coronary heart disease. A tight glycaemic control reduces the morbidity and mortality associated to type 2 diabetes [1;2], but has proven challenging and is usually not sustained. The currently used anti-diabetic drugs show a loss of efficacy over time [3], a poor tolerability and low compliance due to numerous adverse effects, including severe hypoglycaemia, weight gain, oedema, nausea and gastrointestinal derangements. Thus, new strategies were needed that allow a sustained glycaemic control and avoid hypoglycaemia and other side effects.

After food ingestion, specialized neuroendocrine cells of the gastro-intestinal tract release peptides which act to improve glucose handling and energy homeostasis. Among these gut hormones are the incretins glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP) which increase meal-stimulated insulin release

by the pancreas, suppress glucagon secretion and improve glucose disposal. Recent advances in the understanding of the physiological functions of incretins have led to the emergence of a new class of oral anti-diabetic drugs which, by inhibiting the dipeptidyl peptidase (DPP)-4 enzyme, result in increased concentrations of the endogenous incretins GLP-1 and GIP, and consequently improve fasting and post-prandial hyperglycaemia. Several DPP-4 inhibitors have been developed, with five already approved in the USA, Europe and/or Japan (sitagliptin, vildagliptin, saxagliptin, alogliptin and linagliptin). These inhibitors display different chemical structures and pharmacodynamic/pharmacokinetic properties, but all are efficacious for managing diabetes and generally well-tolerated. They allow a reduction in HbA1c levels by 0.5 to 1%, and have other beneficial actions on pancreatic β-cell function, blood pressure and lipid levels for instance.

The 'incretin effect': physiological basis

The term 'incretin effect' has been coined to describe stimulated insulin secretion after oral glucose ingestion compared to isoglycaemic parenteral or iv glucose administration [4]. Several peptides released by the intestine into the circulation in a nutrient-dependent manner account for this potentiation of post-prandial insulin release by the pancreas, and act as triggering signals for the control of appetite, intestinal nutrient absorption and ultimately in the maintenance of energy homeostasis [5]. GIP, for instance, is synthesized and released by entero-endocrine K cells present in the duodenum in response to nutrient (glucose or fat) ingestion and enhances glucose-stimulated insulin secretion [6;7]. The other incretin hormone GLP-1 derives from a pro-glucagon precursor peptide that gives rise also to GLP-2, oxyntomodulin and glicentin [8]. GLP-1 is secreted by L-cells mainly of the distal small intestine (ileum) and colon. Infusion of GLP-1 in the fasted state to mimic its post-prandial concentration increases insulin biosynthesis and release, and diminishes blood glucose [9;10]. Plasma levels of both peptides are low in the fasted state, rise within minutes after food ingestion and contribute to lower blood glucose by exerting potent glucose-dependent insulin stimulatory actions [7]. Indeed, GLP-1 and GIP stimulate β-cell insulin secretion [11;12]. In addition, the two incretins regulate pancreatic islet neogenesis, proliferation and survival in vitro [13] (**Figure 1**).

Beside its insulinotropic effect, GLP-1 inhibits glucagon secretion by the pancreatic α-cells [14], resulting in a further decrease of hepatic glucose production in the post-prandial state in subjects treated with GLP-1-receptor agonists or DPP-4 inhibitors. GLP-

1 also potentiates glucose disposal. Moreover, GLP-1 reduces food intake and gastric emptying through either direct effects on the gastrointestinal tract or indirect actions via the central nervous system [13], promoting satiety and improving weight maintenance. It is worth noting that, in humans, these effects are observed at supraphysiological concentrations, ie. only after administration of GLP-1 analogs. In addition, GLP-1 inhibits intestinal lipoprotein secretion and may lower post-prandial hyperlipidaemia, which is a cardiovascular risk factor. Finally, GLP-1 exerts potentially beneficial vascular and cardio-protective actions (see below). In support of the gluco-regulatory and insulin stimulatory actions of GLP-1, GLP-1 receptor-deficient mice display altered glucose tolerance and diminished glucose-stimulated insulin secretion, abnormal pancreatic islet number and size, but exhibit normal insulin sensitivity and glucagon secretion [15].
As mentioned above, GIP promotes insulin secretion in response to glucose. However, it does not inhibit glucagon secretion nor does it modulate gastric emptying [13]. In addition, GIP increases fatty acid uptake and lipogenesis by adipocytes [16;17], promoting fat deposition. Accordingly, GIP-receptor-deficient mice have defective insulin secretion in response to oral glucose [18], but resist to the development of diet- or genetically-induced obesity and have improved insulin sensitivity [19]. GLP-1-receptor and GIP-receptor double knock-out mice are glucose intolerant as shown by the abnormal glucose excursion in oral glucose tolerance tests, and significantly lower glucose-stimulated insulin secretion [20].

Several reports suggest that the incretin effect is reduced in type 2 diabetic patients [21] [22], although recent studies indicate that the reduction of insulin secretion is primarily due to an impairment in β-cell function after chronic hyperglycaemia rather than a primary defect in glucose-dependent GLP-1 and GIP action [23]. Defective post-prandial GLP-1 secretion in a mixed meal test has been observed in subjects with impaired glucose tolerance, albeit to a lesser extent compared to diabetic patients [22;24;25], although conflicting data have also been published [26]. In addition, the incretin effect and postprandial GLP-1 response is reduced with increasing obesity [27;28]. However, although its secretion is altered, GLP-1 action is mostly preserved in type 2 diabetic patients, fostering pharmaceutical efforts aimed to potentiate or prolong GLP-1 action. Unlike GLP-1, circulating levels of GIP are not diminished in type 2 diabetic patients [22], and GIP has reduced insulinotropic actions in type 2 diabetes, which contributes to the reduced incretin effect in diabetics thus making it a much less attractive therapeutic target.

GLP-1 is secreted as GLP-1(7-36)-amide, the major circulating GLP-1 form, and GLP-1(7-37). It is released within minutes of food ingestion and displays a short half-life (~1-2 min) [29] because of its rapid degradation through the action of DPP-4 and its renal clearance. Only 25% of the secreted GLP-1 reaches the portal vein, and only 10 to 15% the general circulation (**Figure 2**). The half-life of administered GIP is 5 and 7 minutes in diabetic and non-diabetic subjects, respectively [30]. To counteract this rapid cleavage of native GLP-1 (and GIP), two therapeutic approaches have been developed: GLP-1 mimetics resistant to the degradation by DPP-4 and inhibitors of DPP-4 (or gliptins).

Physiological and preclinical studies to support the use of DPP-4 inhibitors in the control of glucose homeostasis

DPP-4 exists as a cell surface membrane-bound peptidase which is expressed in many tissues including the gastro-intestinal tract, liver, kidney, the vascular epithelium and the exocrine pancreas, and conveys intracellular signals to transduction pathways. DPP-4 also exists and exerts enzymatic activity in the plasma where it preferentially cleaves peptides with a proline or an alanine in position 2 of the N-terminus of the peptide. Hence, both GLP-1 and GIP, among others, are endogenous substrates for DPP-4. DPP-4 inhibition (by gliptins) or deletion studies in animal models have shed light on the role of this enzyme in glucose control. DPP-4-deficient mice show improved glucose tolerance, higher plasma GLP-1, GIP and insulin levels following an oral glucose gavage, reduced food intake and increased energy expenditure; they are resistant to diet-induced obesity and insulin resistance and to streptozotocin-induced diabetes [31;32]. Consistently, DPP-4 inhibition improved glucose tolerance, diminished hyperglycaemia and increased insulin secretion in diabetic Zucker rats [33-35] and increased hepatic and peripheral insulin sensitivity [36]. In vitro, vildagliptin-mediated DPP-4 inhibition increased insulin secretion by pancreatic islet cells [37]. Consistently, vildagliptin administration led to decreased weight gain and food intake, increased insulin levels and increased β-cell mass in carbohydrate-fed rats [38]. Sitagliptin treatment in vivo improved glucose homeostasis and pancreatic β-cell survival in diabetic streptozotocin-treated mice [39]. Sitagliptin has also been shown to increase β-cell number and insulin content [40;41]. Treatment with a sitagliptin analog (des-fluoro-sitagliptin) of diabetic ICR mice significantly reduced HbA1c and glucose levels, and decreased liver lipid accumulation while increasing pancreatic insulin content and enhancing glucose-stimulated insulin secretion [42].

In vitro, many other peptides are degraded by DPP-4 including GLP-2, growth-hormone-releasing hormone, pituitary adenylate cyclase activating polypeptide (PACAP) [43]. However, the hypoglycaemic action of DPP-4 inhibitors has been attributed mainly to increased GLP-1 and GIP levels. Indeed, administration of LAF237 (later renamed vildagliptin) or valine-pyrrolidide to inhibit DPP-4 activity led to a reduction of fasting glucose levels and ameliorated glucose tolerance in wild-type, but not in GLP-1-receptor and GIP-receptor double mutant mice, suggesting that both GLP1 and GIP signalling are necessary to the hypoglycaemic action of DPP-4 inhibitors [20]. In addition, administration of LAF237 to high-fat diet-fed mice improved glycaemic control in wild-type, but not GLP-1R and GIPR double mutant mice [44].

Pharmacodynamic and pharmacokinetic properties of the different DPP-4 inhibitors

As mentioned above, several inhibitors are on the market or in trials (**Table 1**). They are all orally available and well absorbed (ie significant DPP-4 inhibition is observed as soon as 15 minutes after administration), and have high affinity for DPP-4. They all potently inhibit plasma DPP-4 to similar levels, but with different IC50 (ie the concentration needed to achieve 50% of inhibition) ranging from 1nM for linagliptin, 19 and 24nM for sitagliptin and alogliptin, to 50 and 62nM for saxagliptin and vildagliptin, respectively. Their half-life also is different, leading to different dosing amount and frequency as outlined below.

Sitagliptin is well absorbed (~87%), and potently and selectively inhibits DPP-4. Its pharmacokinetic and pharmacodynamic properties have been determined in randomized, double-blind, placebo-controlled studies with single oral doses (1.5-600 mg) in healthy subjects [45]. Sitagliptin has an apparent terminal half-life ranging from 8 to 14 hours. Single doses of sitagliptin markedly and dose-dependently inhibited plasma DPP-4 activity, with $\geq$80% inhibition over the entire 24-hour period at 100 mg. PK-PD relationships using multiple oral doses of sitagliptin were also obtained in healthy volunteers [46] and showed similar plasma concentrations, Tmax (time to maximal plasma concentration), Cmax, and terminal half-life (11.8 to 14.4 hours) at day 1 (ie after a single dose) and day 10 (steady-state) of treatment. In this study, plasma DPP-4 activity was inhibited by more than 80% over 24 hours at 50 mg and higher doses. GLP-1 concentrations were increased by approximately 2-fold by sitagliptin. In obese, non-diabetic subjects, sitagliptin 200 mg bid was well tolerated and led to approximately 90%

inhibition of plasma DPP-4 activity, increased GLP-1 levels by 2.7-fold, and decreased post-oral glucose tolerance test glucose excursion by 35% compared to placebo [47].

Vildagliptin is also well (85%) and rapidly absorbed (within 1 to 2 hours) [48]. Vildagliptin is quickly cleared from plasma, with a half-life of 1.5 to 4.5 hrs, thus demanding higher dosing frequency (twice daily vs once daily for other gliptins). PD-PK relationships have been studied in a cross-over, placebo-controlled study in type 2 diabetic patients using doses ranging from 10 and 25 to 100mg twice a day, for 28 days [49]. More than 90% inhibition of DPP-4 activity was observed at all doses, and more than 80% 12 hrs post-dosing [50].

Saxagliptin displays a good bioavailability (67%) and is also a potent selective long-acting DPP-4 inhibitor administered usually at a dose of 5mg/day once a day [51]. As vildagliptin, it is cleared rapidly from the plasma with a ½ life of 2.5hrs, and between 3 and 7 hours for its major 5-hydroxy saxagliptin metabolite (BMS-510849), also a reversible inhibitor of DPP-4 but 2-fold less potent than the parent molecule. However, it is administered once daily, based on the reasoning that saxagliptin, as vildagliptin, forms a covalent, yet reversible, complex with the enzyme, which has been suggested to confer an extended activity, whereas sitagliptin, alogliptin and linagliptin form non-covalent bounds.

The PK-PD relationship of various doses (25, 100 or 400 mg) of alogliptin was studied in type 2 diabetic patients over 14 days [52]. A potent inhibition of plasma DPP-4 activity (82-97%) was observed at 24 hrs after administration of the last dose [53]. Alogliptin is usually used at 25 mg once a day.

Linagliptin is generally used at a dose of 25 mg once a day. It inhibits DPP-4 *in vitro* with a lower IC50 ~ 1nM compared to other gliptins. Linagliptin has been administered to healthy volunteers at different doses (up to 600mg). It is well tolerated, with a low renal excretion and a terminal half-life around 180 hours [54]. Single doses of 2.5 mg and 5 mg inhibited DPP-4 activity by 72.7% and 86.1%.

Potency and duration of inhibition

As outlined above, although some differences exist with regards to their chemical structures and pharmacodynamics-kinetics, when administred at therapeutic doses (from 5 to 100 mg) and dosing frequencies (once/twice a day), they all potently inhibit plasma DPP-4 activity (measured ex vivo) by 70 to 90% at 24 hours post-dosing. Indeed, a single dose of sitagliptin (100mg) inhibited plasma DPP-4 activity by $\geq$80% over a 24-hour period [46], while the same dose of vildagliptin inhibited by more than 80% DPP-4

activity 12 hrs post-dosing [50]. Similar results were obtained in healthy and type 2 diabetic patients, with 25 mg alogliptin or 5 mg linagliptin, with more than 80 % inhibition over 24 hrs [53-55]. Thus, all DPP-4 inhibitors exert potent and sustained DPP-4 inhibition at therapeutic dosing.

Metabolism and excretion

Sitagliptin is mainly (74%) eliminated unmodified via the kidney in a dosage-independent manner, and renal insufficiency may cause an increase in circulating levels [56;57] requiring different dosing in these patients. A single dose of 50 or 25 mg sitagliptin has been administered daily to type 2 diabetic patients with renal insufficiency for 54 weeks [58]. A reduction of HbA1c levels by 0.7% has been observed and adverse events were not increased. Sitagliptin gives rise through the action of hepatic CYP450 to 6 metabolites, 3 being active [57]. As sitagliptin, alogliptin is mainly excreted unchanged in the urine [53]. In contrast, linagliptin is primarily excreted via the bile, and renal impairment has only a minor effect on linagliptin pharmacokinetics. Thus, there will be no need for adjusting the linagliptin dose in renally impaired patients with T2D [59]. By contrast, vildagliptin is mainly (> 50%) hydrolyzed by P450 cytochromes to one major pharmacologically inactive compound which is excreted in the urine. Hence, dose adjustment may apply in renal or hepatic insufficiency. Saxagliptin is metabolized in the liver via P450 cytochromes to an active compound which is also a competitive reversible DPP-4 inhibitor. Both this compound and the parent saxagliptin are cleared by the kidney. However, hepatic insufficiency does not seem to alter the pharmacokinetics of saxagliptin [60].

Selectivity

DPP-4 belongs to a large family of structurally related prolyl-peptidases which includes also DPP-8 and DPP-9. Lankas et al. have shown that DPP-8/9 inhibition in vivo in rats leads to alopecia, thrombocytopenia, reticulocytopenia, enlarged spleen, multiorgan histopathological changes, and mortality [61]. Non- or partial selectivity of DPP-4 inhibitors could therefore be of clinical importance although a class effect is very unlikely because of the differences in the chemical structure of the different DPP-4 inhibitors. Hence, molecules thought to be specifically DPP-4-targeted may in fact inhibit other enzymes of this family and its selectivity should be carefully examined. Sitagliptin, alogliptin and linagliptin are highly selective of DPP-4, whereas vildagliptin appears less

selective. However, in mice and rats, chronic administration of vildagliptin at doses allowing DPP8/9 inhibition did not result in organ toxicity [62].

DPP-4 inhibitors in clinical trials

Although structurally different, all DPP-4 inhibitors efficiently lower fasting and post-prandial hyperglycaemia and reduce HbA1c levels by 0.7 to 1% as shown in numerous phase II and phase III trials. They also improve β-cell function in short-term studies as attested by the HOMA-B index and the decreased pro-insulin/insulin ratio.

Sitagliptin

Sitagliptin has been evaluated in several phase III trials as monotherapy or combination therapy. As monotherapy, in a placebo-controlled, double-blind, 24 weeks study, sitagliptin 100mg and 200 mg led to a reduction in HbA1c (-0.79 and -0.94%, respectively) and fasting glucose (-17.1 mg/dl and -21.3 mg/dl, respectively) levels, and lower post-prandial glucose in a meal tolerance test [63]. A greater reduction of HbA1c was achieved in patients with baseline HbA1c≥9% (placebo-subtracted reductions of -1.52 and -1.50% for the 100- and 200-mg treatment groups, respectively). At 24 weeks, the percentage of patients achieving HbA1c<7% was 41% with 100mg and 45% with 200mg vs 17% for placebo. In this study, sitagliptin also improved the HOMA-B index without clinically relevant hypoglycaemic episodes and with a small but significant weight loss compared to placebo.

In a double-blind, randomized study, sitagliptin 100 mg/d alone or in combination with 2g/d metformin during 24 weeks reduced HbA1c levels by 0.83% and 2.07% vs placebo, respectively, demonstrating an additive effect [64]. Adverse effects were similar to those with metformin alone. In another study, sitagliptin 100mg has been given in association with metformin vs metformin alone, for 24 weeks. Sitagliptin reduced HbA1c levels by an additional 0.65% [65]. Fasting blood glucose was also reduced by 25.4 mg/dl, HOMA-B was improved and body weight remained unchanged. A small but significant decrease in plasma cholesterol (total- and non-HDL-cholesterol) and triglycerides was also observed with sitagliptin 100mg but the between-group (placebo-subtracted) differences were not significant.

Sitagliptin (100mg once daily) in association with pioglitazone 30 or 45mg/d was evaluated in a 24-week study. It reduced HbA1c levels by 0.85% (baseline HbA1c=8.1%), fasting blood glucose by 16.7mg/dl, and after 24 weeks 45% of the patients had HbA1c less than 7% vs only 23% taking pioglitazone alone [66].

Sitagliptin was also evaluated for 52 weeks in a non-inferiority trial compared with a sulfonylurea (glipizide: 5 to 20 mg/d) in diabetic patients with inadequate control of HbA1c by metformin alone [67]. Sitagliptin was proven non-inferior on HbA1c (-0.67% at 52w) and glucose levels, with significantly less hypoglyceamic episodes and a gradual decrease in body weight compared to glipizide.

Similarly, in another study comparing the addition of sitagliptin or glipizide on on-going metformin therapy during 2 years, sitagliptin reduced HbA1c levels by -0.54% compared to -0.51% with glipizide and the percentage of patients with HbA1c levels <7% was 63% and 59%, respectively [68]. Sitagliptin was associated with weight loss (-1.6 kg) and a lower incidence of hypoglycemia.

In a recent head-to-head comparison of sitagliptin vs liraglutide, a GLP-1 analog, in patients with inadequate glycaemic control on metformin (mean HbA1c 8.5% at baseline) [69], 100 mg sitagliptin for 26 weeks reduced HbA1c levels by 0.9%, whereas 1.2mg and 1.8 mg liraglutide led to a significantly greater reduction (-1.24% and -1.50%, respectively). In addition, mean weight loss was greater with liraglutide compared to sitagliptin, whereas nausea was more common with liraglutide than with sitagliptin.

Vildagliptin

Used as a monotherapy for 24 weeks in a double-blind, placebo-controlled study in drug-naïve diabetic patients, vildagliptin (50mg/d, 50mg twice a day, or 100mg qd) significantly reduced HbA1c by 0.5%, 0.7% and 0.9%, respectively, and reduced fasting glucose levels without change in body weight and the incidence of adverse events [70].

Vildagliptin 50mg once or twice daily in association with metformin for 24 weeks was administered to diabetic patients with baseline HbA1c levels ~ 8.4%. Vildagliptin 50 and 100mg reduced HbA1c by -0.7 and -1.1%, respectively. Fasting (-14 and -31mg/dl) and post-prandial glucose (-34 and -41mg/dl) was also decreased [71]. In association with pioglitazone (45 mg daily) in diabetic patients with inadequate glycaemic control on prior monotherapy, vildagliptin (50mg once or twice daily for 24 weeks) resulted in a decrease of HbA1c levels by -0.8 and -1%, fasting glucose levels by -14.4 and -19.8 mg/dl, and reduced post-prandial glucose [72]. In both studies, no change in body weight was observed, and vildagliptin was well tolerated (the incidence of hypoglycemia and other adverse effects was not increased).

Vildagliptin 50 mg twice daily vs sulphonylurea (glimepiride) was administered for 52 weeks in patients receiving metformin and displaying inadequate glycaemic control [73]. Vildagliptin treatment reduced HbA1c and fasting glucose levels by -0.44% and -18mg/dl

and was non-inferior compared to glimepiride (mean dose: 4.5 mg/d). The incidence of hypoglycemic episodes was significantly reduced in the vildagliptin arm compared to the sulphonylurea treated group.

Linagliptin

Linagliptin was also evaluated as monotherapy or add-on therapy and has shown clinically meaningful improvement of glycaemic control in type 2 diabetes. Linagliptin was given as monotherapy, 5mg once daily achieving a concentration $\geq$6.4nM leading to a median >82% DPP-4 inhibition, during 24 weeks in drug-naïve patients or patients receiving oral antidiabetic agents (other than TZDs) prior to a 6-week wash-out period [74]. In this multicenter, randomized, placebo-controlled, phase III trial, linagliptin improved glycaemic control (HbA1c -0.69 % from baseline, -1.01% in the patients with a baseline HbA1c $\geq$ 9%). The percentage of patients who achieved HbA1c<7% was 25.2% with linagliptin vs 11.6% in the placebo group. Fasting and 2h post-prandial glucose were significantly reduced in the linagliptin arm compared to placebo (-1.3 mM [23mg/dl] vs placebo and -3.2 mM [58mg/dl] vs placebo, respectively). Glucose excursion after a test meal was also significantly reduced. In addition, an enhancement of β-cell function was observed (differences were seen in the proinsulin/insulin ratio, the HOMA-B index and the disposition index). Linagliptin was well tolerated and the safety profile was comparable to the placebo group (no drug-related serious event) and hypoglycaemic episodes were not increased compared to the placebo group. There was no increase of body weight.

The efficacy and safety of linagliptin (5 mg once daily) in combination with metformin during 24 weeks was also evaluated in type 2 diabetic patients in a randomized, placebo-controlled, double-blind, multi-center study [75]. Linagliptin led to a significant reduction in HbA1c (-0.5% from baseline) and fasting and post-prandial glucose. The risk of hypoglycaemic episode was not increased and linagliptin was weight neutral. As mentioned above, linagliptin is mainly excreted unchanged in the urine and can thus be used in patients with severe renal insufficiency [76].

Linagliptin (5mg once daily) has proven non-inferior to sitagliptin (100 mg once daily) in T2D patients in a randomized, double-blind, placebo-controlled study assessing fasting and 24-hrs plasma glucose changes from baseline as well as plasma GLP-1 AUC (0-2h) and glucose AUC (0-3h) from baseline (clinicaltrials.gov, NCT00716092).

Alogliptin

Clinical evidence also supports the efficacy of alogliptin. Used as a monotherapy in a double-blind placebo-controlled study, alogliptin 12.5 and 25mg once daily resulted in a significant reduction of HbA1c by 0.6%, and was weight neutral [77].

Several studies showed that alogliptin in combination with other oral antidiabetic agents also improves glycaemic control. In association with metformin in type 2 diabetic patients with baseline HbA1c from 7 to 10%, 12.5 and 25mg alogliptin once daily for 26 weeks significantly reduced HbA1c and fasting blood glucose by an additional -0.6% and -17mg/dL, respectively, over placebo [78].

In a randomized, double-blind trial, the administration of 12.5 and 25mg alogliptin for 26 weeks in patients inadequately controlled by the sulphonylurea glyburide (mean baseline HbA1c 8.1%) led to a further reduction of HbA1c levels by 0.53 and 0.39%, and fasting glucose by -8.4 and -4.7mg/dL [79].

Used with insulin, alogliptin 12.5 and 25 mg for 26 weeks improved glycaemic control as Hb1Ac was decreased by 0.63% and 0.71%, respectively [80].

Saxagliptin

Saxagliptin also improves glycemic control and is well-tolerated. As monotherapy, saxagliptin 2.5, 5, 10, 20 and 40 mg once a day reduced HbA1c levels by 0.45-0.63% vs placebo and was neutral on body weight [81;82]. In another study, saxagliptin 2.5, 5 and 20mg daily for 24 weeks led to a reduction of HbA1c of 0.43%, 0.46% and 0.54%, respectively [81;82].

Saxagliptin vs placebo added to either metformin, a TZD (rosiglitazone or pioglitazone) or glyburide for 24 weeks resulted in significant reductions in HbA1c (-0.69%, -0.94% and -0.64% at 5mg) [83-85]. Fasting glucose was also reduced. The incidence of hypoglycaemia and other adverse events was not different from placebo in either three studies. In a randomized, double-blind, placebo-controlled study, saxagliptin was given for 12 weeks at 2.5 mg once daily to patients with renal impairment and inadequately controlled type 2 diabetes (baseline HbA1c 7 to 11%) [86]. In this study, saxagliptin lowered HbA1c by 0.42% over placebo, and achieved greater HbA1c reduction than placebo in a subset of patients with moderate (-0.64% vs -0.05%) and severe (-0.95 vs -0.50%) renal insufficiency.

Saxagliptin 5 mg once daily and sitagliptin 100 mg once daily were compared in a head to head, 18-week, double-blind, non-inferiority trial in patients on metformin with inadequately controlled glycaemia [87]). The mean changes in HbA1c in the saxagliptin

and sitagliptin groups were -0.52 and -0.62%, respectively. There was no between-group difference, demonstrating non-inferiority of saxagliptin vs sitagliptin. Both treatments were equally tolerated.

Adverse effects

DPP-4 inhibitors are generally well-tolerated, and no increase in adverse events was noted compared to placebo or other comparatives, but again slight differences may exist between the different molecules of this class. DPP-4 is also present on the cell membrane of T lymphocytes known as CD26. In these cells, it acts by activating intracellular signalling pathways to simulate T cell proliferation. In pre-clinical models, DPP-4-deficiency results in modest abnormalities in immune response, decreased CD4+ T cell number, and reduced production of interleukin (IL)-4 while IL-10 was increased [88;89]. However, the peptidase activity of DPP-4 has not been associated to immune function. DPP-4 inhibitors did not affect immune function in rats and dogs [61]. The effect of sitagliptin was also examined in type 1 diabetes where it was shown to reduce the effect of autoimmunity on islet graft survival linked to decreased T cell migration [90]. In the same line, alogliptin was shown to suppress LPS-induced TLR-4 signalling [91]. Saxagliptin administration led also to a modest reduction in lymphocyte count within the normal range [81]. DPP-4 inhibition may also affect human progenitor cell and hematopoietic stem cell migration [92]. To date, no adverse events related to immunological effects have been reported in humans but additional long-term trials are needed before to conclude on their safety profile with regards to immunological issues.
Some infections of the urinary tract and of the upper respiratory tract (nasopharyngitis) were reported to be slightly increased with the use of sitagliptin and vildagliptin, but this was not confirmed in later studies [93].

As mentioned above, several hormones and peptides harbouring an alanine or a proline at position 2 are degraded by DPP-4 in in vitro systems [43], although only a few were identified as endogenous physiological substrates. These include the chemokines SDF1α and β, GLP-2 and the vasodilatator substance P. DPP-4 inhibitors are considered safe therapies, and no serious adverse events have been reported. Nevertheless as with all new drug classes, long-term follow up is required to evaluate potential safety issues.

DPP4 inhibitors and cardiovascular risk

Numerous studies have evidenced a role for GLP-1 in the cardiovascular system. On the one hand, continuous IV administration of GLP-1 in cardiac insufficiency and myocardial infarction improves cardiac function by increasing left ventricular ejection fraction [94]. Administration of the DPP-4-resistant GLP-1 analog exendin-4 to apoE-deficient mice, which spontaneously develop atherosclerosis, diminished atherosclerotic lesion size and monocyte/macrophage recruitment to the vascular wall independent of changes in plasma lipid levels or glucose tolerance [95]. On the other hand, deletion of the GLP-1 receptor in mice results in increased left ventricular diastolic pressure, decreased left ventricle (LV) contractility and increased LV thickness [96]. In humans, GLP-1 administration increased left ventricular ejection fraction and improved the resistance to exercise in diabetic and non-diabetic patients with cardiac insufficiency [97], suggestive of a potential cardioprotective effect of GLP-1. In the same line, GLP-1 infusion lowered arrhythmia in patients with coronary artery bypass grafting [98], while retrospective data base analysis suggested that administration of exenatide may diminish the relative risk of CVD in type 2 diabetic patients [99]. By contrast, 48-h GLP-1 infusion had no major cardio-vascular effects in patients with congestive heart failure [100].

A recent study conducted in 14 patients with coronary artery disease indicated that a single administration of 100mg sitagliptin increases ejection fraction and improves contractile function on ischemic segments [101]. It is noteworthy that not all effects are necessary dependent on DPP-4 as sitagliptin was shown to slightly improve recovery from ischemia-reperfusion in isolated hearts from DPP-4 deficient as well as wild-type mice [102]. In addition, sitagliptin was shown to reduce diastolic blood pressure and heart rate with a similar extent than liraglutide [69]. A meta-analysis has suggested a potential reduction of cardiovascular events with saxagliptin [103]. A meta-analysis of 29 placebo-controlled and 11 active comparator, randomized trials with DPP-4 inhibitors (sitagliptin and vildagliptin) also found a reduction in the risk of cardio-vascular events (odd ratio=0.76 vs control groups) [104]. Thus, evidence so far indicates that gliptins do not adversely affect the risk of cardiovascular events and animal studies suggest potential protective effects. However, prospective trials investigating the effects of DPP-4 inhibitors on cardiovascular outcomes are ongoing (**Table 2**) and the results eagerly awaited. TECOS (clinicaltrials.gov, NCT00790205) is a randomized, placebo-controlled clinical trial designed to assess the cardiovascular outcome of long term (up to 5 years) treatment with sitagliptin in an estimated number of 14,000 patients with T2D (HbA1c between 6.5% and 8.0%) and a history of cardiovascular disease. Primary end-points

are defined as CV-related death, nonfatal myocardial infarction (MI), nonfatal stroke, or unstable angina requiring hospitalization ([105] and http://clinicaltrials.gov/). The cardiovascular safety of linagliptin will be tested in a randomised, double-blind study in 6,000 patients with T2D and at elevated cardiovascular risk. Linagliptin will be compared against glimepiride (CAROLINA study)[106]. The primary outcome is defined as the time to the first occurence of any of the following events: CV death, non-fatal MI, non-fatal stroke and hospitalisation for unstable angina pectoris (clinicaltrials.gov, NCT01243424). SAVOR-TIMI 53 (NCT01107886) is a randomised, double-blind, placebo-controlled phase IV trial evaluating the effect of saxagliptin on the incidence of cardiovascular death, non-fatal MI or non-fatal ischaemic stroke in 16,500 patients with T2D. Finally, EXAMINE (NCT00968708) is a randomized, double-blind, placebo-controlled study that will evaluate cardiovascular outcomes upon treatment with alogliptin in subjects with T2D and acute coronary syndrome (acute MI or unstable angina)[107]. In addition, the effects of 52 weeks vildagliptin treatment on LV function will be tested in patients with T2D and congestive heart failure (NCT 00894868) in a randomized, double-blind, placebo-controlled phase IV trial (estimated completion date jan 2013).

Conclusion

DPP-4 inhibitors are a novel class of orally available molecules for the treatment of type 2 diabetes. Although structurally different, they share a common mechanism of action by extending the half-life of endogenous GLP-1 thus prolonging its actions, they potently reduce blood glucose levels and lower HbA1c by up to 1%. They are generally well tolerated and safe. Because GLP-1 is secreted in a glucose-dependent manner, DPP-4 inhibitors, which prolong its half-life, are not associated with an increased risk of hypoglycaemic episodes.

Table 1: Gliptins in development and licensed.

Compound	**Company**	**Status**
Sitagliptin (MK-0431)	Merck	Launched 2006
Vildagliptin (LAF-237)	Novartis	Launched 2008
Saxagliptin	Bristol-Myers Squibb and Astra Zeneca	Launched 2009; recently approved in Europe for the treatment of T2D with moderate to severe renal insufficiency or mild hepatic insufficiency (2011)
Alogliptin (SYR-322)	Takeda	Launched in Japan
Linagliptin (BI-1356)	Boehringer-Ingelheim	Launched
Dutogliptin (PHX1149)	Phenomix Pharmaceuticals	Phase III
Teneligliptin (MP-513)	Mitsubishi Tanabe Pharma Corp	Phase III, filled application for Japan (sept 2011)
SYR472	Takeda	Phase III
KRP104	Kyorin	PhaseII
LC15-0444	LG Life Sciences	Phase II
Melogliptin (GRC8200)	Glenmark	Phase II

Table 2: Ongoing trials investigating the effects of DPP-4 inhibitors on cardiovascular disease outcomes

Trial	Intervention and duration	Inclusion and estimated nb of patients	Primary endpoint	Estimated completion date / Status
TECOS (Phase III) NCT00790205 *	Sitagliptin vs placebo (Up to 5 years)	14,000 subjects with T2D and inadequate glycemic control and history of CVD, >50y	CV-related death, nonfatal MI, nonfatal stroke, or unstable angina requiring hospitalization	Dec 2014/ Recruiting
CAROLINA (Phase III) NCT01243424	Linagliptin vs glimepiride 400 weeks (7.5 years)	6,000 T2D patients	CV death, non-fatal MI, non-fatal stroke and hospitalisation for unstable angina pectoris at Week 400 (or FinalVisit)	Sept 2018/ Recruiting
SAVOR-TIMI 53 (Phase IV) NCT01107886	Saxagliptin vs placebo (5 years)	16,500 T2D patients	CV death, non-fatal MI or non-fatal ischaemic stroke	April 2014/ Recruiting
EXAMINE (Phase III) NCT00968708	Alogliptin vs placebo (4.7 years)	5,400 T2D patients with acute coronary syndrome	CV, nonfatal MI and nonfatal stroke	dec 2014/ Recruiting

* clinicaltrials.gov identifier

Legends to figures

Figure 1: Physiological actions of GLP-1 in different organs/tissues

Upon meal ingestion, GLP-1 is secreted by the intestine. GLP-1 enhances glucose-stimulated insulin secretion ('incretin effect'), diminishes glucagon production and hepatic glucose production, and increases pancreatic β-cell proliferation and survival. Additional actions have been described including gastric emptying, reduction of appetite and cardioprotective actions, among others.

Figure 2: GLP-1 is rapidly degraded by dipeptidyl-peptidase (DPP-4)

GLP-1 has a short half-life (1-2 min) because of rapid proteolytic degradation by DPP-4 (and renal elimination). DPP-4 inhibitors thus increase its half-life which results in prolongation of its biological actions.

Figure 3: Structure of the different approved DPP-4 inhibitors.

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